The Immunocytochemical Distribution of Leukocytic Subpopulations in Human Endometrium

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Thirty human endometria were selected from women aged 21–54 years who had undergone routine dilation and curettage procedures for tubal ligation, infertility dating, and irregular menstrual cycling. Histologic sections of the cases chosen were examined to exclude any major pathologic condition (including chronic endometritis). The specimens were stained with monoclonal antibodies to a common leukocytic antigen (H Leu-1 and PD7/26), pan-T-cell antigen ($UCHT_1$), T helper/inducer and T suppressor/cytotoxic antigens (Leu-3a and $UCHT_4$, respectively), pan-B cell antigen (To15 and Leu-12), and macrophage antigens ($UCHM_1$ and Leu-M_3). Other antibodies used included TAL-1B5 (anti-HLA-DR), Leu-7 (natural killer cell) and Na 1/34 (anti-T6/Langerhans/interdigitating reticulum cell). The endometria contained significant numbers of common leukocyte antigen-positive cells (occupying approximately 10–15% of the stroma), the numbers of which appeared to increase in the late secretory/premenstrual phase (20–25% of the stroma). The major leukocyte populations were T cells and macrophages; the latter, with neutrophils, appeared to account for the premenstrual increase in leukocytes. T cells were distributed both diffusely in the stroma and in periglandular stromal aggregates closely applied to the glands. The $T8^+$ suppressor/cytotoxic population was predominant within the stromal nodules. In addition, scattered intraepithelial T suppressor/cytotoxic cells were present. Macrophages ($UCHM_1$ and $HLA\text{-}DR^+$) were also distributed diffusely in the stroma and as part of the periglandular stromal aggregates, in areas sending long cell processes into the epithelium. B cells appeared to be limited to scattered cells in the stroma, only increasing in number within lymphoid follicles. Natural killer cells, as defined by $Leu\text{-}7^+$ cells, were also present, scattered singly in the stroma and within lymphoid follicles. The demonstration of large mononuclear dendritic-appearing Na $1/34^+$ cells within the glands of the endometrium in 5/30 cases suggests the presence of $T6^+$ Langerhans/interdigitating reticulum cells in the endometrium. Thus, the normal endometrium has an important population of immunologically competent cells. Further study of these cell populations may elucidate their contribution, if any, to pathologic conditions in the endometrium. (Am J Pathol 1987, 127:66–73)

IT HAS LONG BEEN KNOWN that human endometrium contains leukocytic cells, including lymphoid aggregates, which may be present in up to 50% of endometria from women of reproductive age.[1,2] However, apart from mature granulocytes, lymphocytes, and plasma cells, it is often difficult to determine the nature of leukocytes on routine histologic examination. The use of monoclonal antibodies has made it possible to further define the composition of the leukocytic cell series that are present in the endometrium. The objective of this study was, therefore, to use immunocytochemical markers to assess the distribution of several of the major leukocytic cell series, selecting reasonably normal-appearing cycling and perimenopausal human endometria; postmenopausal endometria were not included.

Materials and Methods

Tissues

Endometrium was selected from 30 patients (ages, 21–54 years), all of whom had undergone routine di-

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lation and curettage procedures for indications such as tubal ligation (18 cases), infertility (4 cases), or irregular menses (8 cases). Tissue obtained at the time of the surgical procedure was either immediately snap-frozen or transported in tissue culture medium and subsequently frozen (within 1 hour) in liquid nitrogen. Tissue for paraffin immunostaining was fixed in acid formalin.[3] Most of the tissue in each case was fixed in 10% formalin for routine histologic sections; the presence of a major pathologic condition was thus excluded prior to immunoperoxidase staining. One frozen section was also stained with Mayer's hematoxylin for exclusion of pathologic lesions on the sample taken for immunoperoxidase study.

Histology

Each case was classified as proliferative, secretory, or menstrual, primarily by referring to the routine formalin-fixed and paraffin-embedded sections. However, the Mayer's hematoxylin-stained cryostat sections were also used for evaluation of the histologic features. Lymphoid follicles were present in 3 cases, all of which consisted of proliferative endometrium.

Immunocytochemistry

The antibodies used, their sources, and specificities are listed in Table 1. Cryostat sections 6 μ in thickness were air-dried at 18–20 C and fixed in fresh acetone for 30 minutes immediately prior to staining with H Leu-1,[4] To15,[5] Leu-12, $UCHT_1$,[6] $UCHT_4$, Leu-3a, $UCHM_1$, Leu-M3,[7] Leu-7, and Na 1/34.[8,9] A double layer staining technique was used involving a primary mouse monoclonal antiserum and a rabbit anti-mouse secondary antiserum conjugated to horseradish peroxidase. The details of this procedure have been described elsewhere.[10] Peroxidase activity was shown with the use of 3′,3′-diaminobenzidine reagent as described by Graham and Karnovsky. Nuclei were counterstained with hematoxylin.

Quantitative Studies of Common Leukocyte Antigen Positive Cells

Quantitative studies of common leukocyte antigen-positive cells in the stroma were carried out on sections stained with H Leu-1. The number of cells recognized as positively stained by this antiserum was counted in three random and consecutive high-power fields with a ×40 objective, and expressed as a percentage of the total number of stromal nuclei counted in the same area.

Results

The results are summarized in Table 2.

Proliferative Endometrium

Many cells showing strong surface staining for common leukocyte antigen (H Leu-1^+ and PD7/26^+) were noted in the stroma and in intraepithelial locations. In the stroma, they accounted for approximately 10–15% of the cells and were distributed either scattered diffusely or in groups applied closely to and, in some cases, appearing to indent the epithelium (Figure 1A). In many instances, comparison with the Mayer's hematoxylin-stained cryostat sections failed to identify these stromal nodules as lymphoid in morphology. However, the use of the acid formalin-fixed

Table 1 — Panel of Monoclonal Antibodies Used

Antibody	Source	Specificity	Dilution
Cryostat sections			
H Leu 1	Dr. P. C. L. Beverley ICRF*	Common leukocyte antigen[4]	Neat
$UCHT_1$	Dr. P. C. L. Beverley ICRF*	Pan-T-cell[6] (anti-T3)	Neat
Leu-3a	Becton Dickinson	T helper/inducer (anti-T4); macrophage	1:500
$UCHT_4$	Dr. P. C. L. Beverley ICRF*	T suppressor/cytotoxic (anti-T8)	Neat
$UCHM_1$	Dr. P. C. L. Beverley ICRF*	Macrophage	Neat
Leu-M3	Becton Dickinson	Macrophage[7]	Neat
Leu-12	Becton Dickinson	Pan-B-cell	1:500
Leu-7	Becton Dickinson	Human natural killer-1	Neat
To15	Dakopatts	Pan-B cell[5]	Neat
Na 1/34	Prof. A. J. McMichael Department of Surgery Oxford University	Langerhans cell/IDRC/ thymocyte (anti-T6)	1:1000
Paraffin sections			
PD7/26	Dr. D. Y. Mason Oxford University	Common leukocyte antigen[13]	Neat
TAL-1B5	I.C.R.F.*	HLA-DR[11]	Neat

*Imperial Cancer Research Fund.

Table 2—Results

Phase	Number of cases	Pattern of distribution
Proliferative	19	1. Common leukocyte antigen positive cells constituted approximately 10–15% of stromal cells. CLA$^+$ cells were present intraepithelially, in periglandular stromal nodules and scattered singly in the stroma. 2. Predominant cells were T cells and macrophages. 3. T cells (T3$^+$) were present both singly in stroma and in stromal nodules closely applied to epithelium. 4. T suppressor/cytotoxic cells (T8$^+$) predominated in the stromal T-cell nodules and appeared to be more plentiful than T helper/inducer cells (T4$^+$). T suppressor/cytotoxic cells were also present intraepithelially. 5. Macrophages (UCHM$_1$ and HLA-DR$^+$) were distributed diffusely in the stroma and as part of the periglandular aggregates. 6. B cells were scattered occasionally in stroma and in lymphoid follicles, when present. 7. Leu-7$^+$ cells were present singly in the stroma and within lymphoid follicles. 8. T6$^+$ cells were scattered in the epithelium in 5 cases.
Secretory	10	Common leukocyte antigen-positive cells, mainly macrophages, increased (20–25% of the stroma) in the late secretory phase.

sections stained with the PD7/26 antibody clearly demonstrated the shrunken and irregular nuclei of the lymphocytes as distinct from the other stromal cells. Both the PD7/26 and the TAL-1B5 antibodies also clearly delineated the periglandular macrophages and their intraepithelial cell processes.

The major cell populations of the leukocyte common antigen-positive cells were T cells and macrophages. T3$^+$ cells (UCHT$_1^+$) showed an overall distribution similar to common leukocyte antigen-positive cells and appeared to form, together with a few macrophages, the stromal nodules closely applied to the epithelium. In addition, intraepithelial T3$^+$ cells were present. In comparing T3 and T8 antigen distributions, it appeared that T8$^+$ cells closely approximated the distribution of T3$^+$ cells (although in lesser numbers), and, in particular, were the predominant population in the periglandular T-cell stromal nodules (Figure 2). T suppressor/cytotoxic cells cells (T8$^+$) were also present singly in the stroma and scattered in the epithelium. T helper/inducer cells (T4$^+$) were seen in the stroma and in small numbers within the groups of epithelium-related T cells; intraepithelial T helper cells were absent. The Leu-3a antibody appeared to cross-react with macrophages; and, thus, its distribution was an overapproximation of the distribution of T helper/inducer cells. Therefore, the UCHT$_1$ and UCHT$_4$ antibody distributions were used in conjunction for more accurate assessment of T-cell populations.

Macrophages (UCHM$_1^+$, Leu-M$_3^+$, and TAL-1B5$^+$) were seen diffusely distributed in the stroma and as part of the stromal nodules in close association with T cells. In some areas, these periglandular macrophages appeared to send cell processes into the glandular epithelium (Figure 3). This arrangement was best seen with the TAL-1B5 antibody. The macrophages showed both surface and cytoplasmic staining for UCHM$_1$ and Leu-M$_3$; however, the cell outlines were poorly delineated.

B cells (To15$^+$ and Leu-12$^+$) were seen only occasionally in the stroma; their numbers increased within recognizable lymphoid follicles where they were strongly positive in the mantle zone of small lymphocytes. Intraepithelial B cells were absent. Natural killer cells (as defined by Leu-7 positivity) were also noted scattered singly in the stroma and in lymphoid follicles. The Leu-7 antibody appeared to stain the glandular lumen and scattered glandular epithelial cells in focal areas.

Rare large mononuclear T6$^+$ (Na1/34$^+$) cells with dendritic-appearing cell processes were seen in the epithelium in 5 cases (Figure 4); however, HLA-DR$^+$ (TAL-1B5$^+$) cells with similar morphology were not seen in these cases.

Secretory Endometrium

The numbers of stromal common leukocyte antigen-positive cells appeared to increase (20–25% of the stroma) in the late secretory phase, predominantly because of an increase in macrophages. All other cells showed distributions similar to those described in the proliferative phase (Figure 1B).

Menstrual Endometrium

Only 1 case studied in this group. There was a massive increase in common leukocyte antigen-positive cells, due mainly to a recognizable increase in neutrophils.

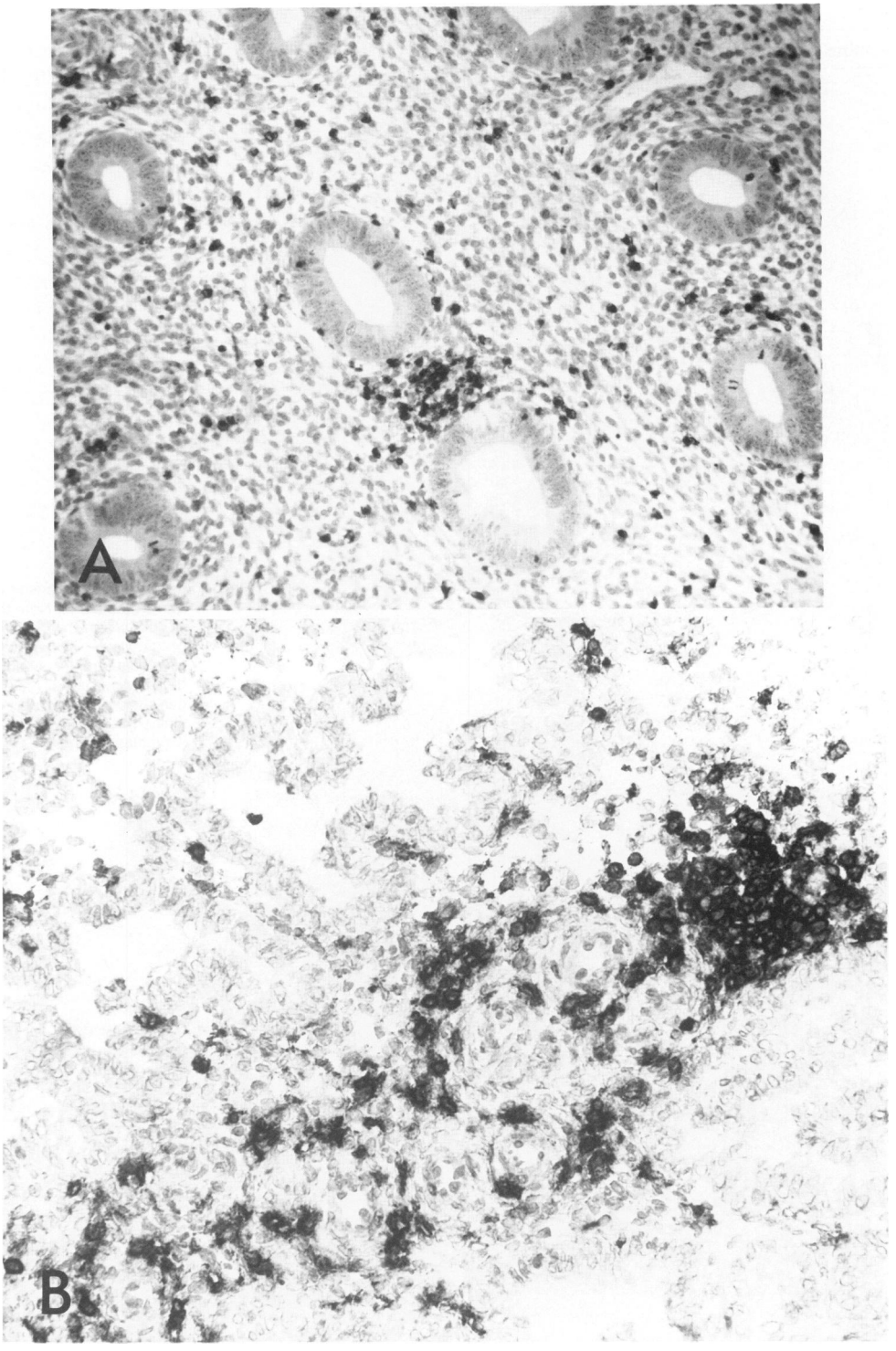

Figure 1—Distribution of common leukocytic antigen-positive cells in both periglandular stromal aggregates and scattered singly in the stroma. **A**—Proliferative endometrium. (Acid formalin-fixed paraffin section, PD 7/26-stained, ×250) **B**—Secretory endometrium. (Cryostat section, H Leu-l–stained, ×300)

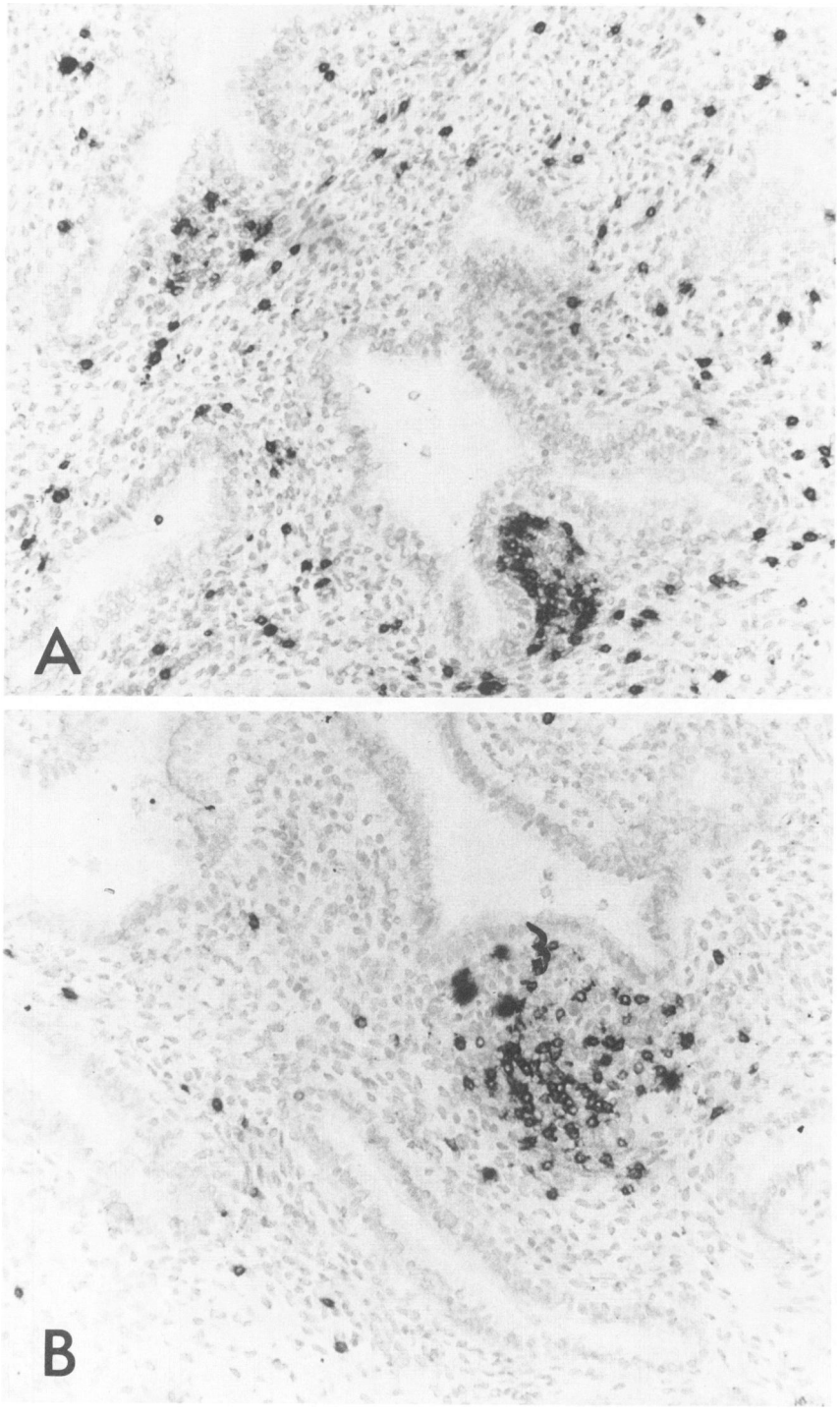

Figure 2—Comparison of T3 and T8 antigen distributions. Note that a large number of cells within the periglandular stromal aggregates were $T3^+$ and that $T8^+$ cells closely approximated the distribution of $T3^+$ cells in these aggregates. (×250) **A**—T3 antigen distribution. (cryostat section, $UCHT_1$-stained) **B**—T8 antigen distribution. (Cryostat section, $UCHT_4$-stained)

Discussion

The presence of leukocytic cells in significant numbers in both proliferative and secretory phases of human endometrium strengthens the concept that this cell population must be regarded as another component of the mucosal-associated lymphoid tissue.[14] The apparent stability of this population throughout the menstrual cycle (at least 10–15% of the stroma at any given time) may contribute to the normal sterile environment of the endometrium. Although some authors describe an increase in lymphocytes during the secretory phase,[15] our results did not support this observation, but rather demonstrated a premenstrual

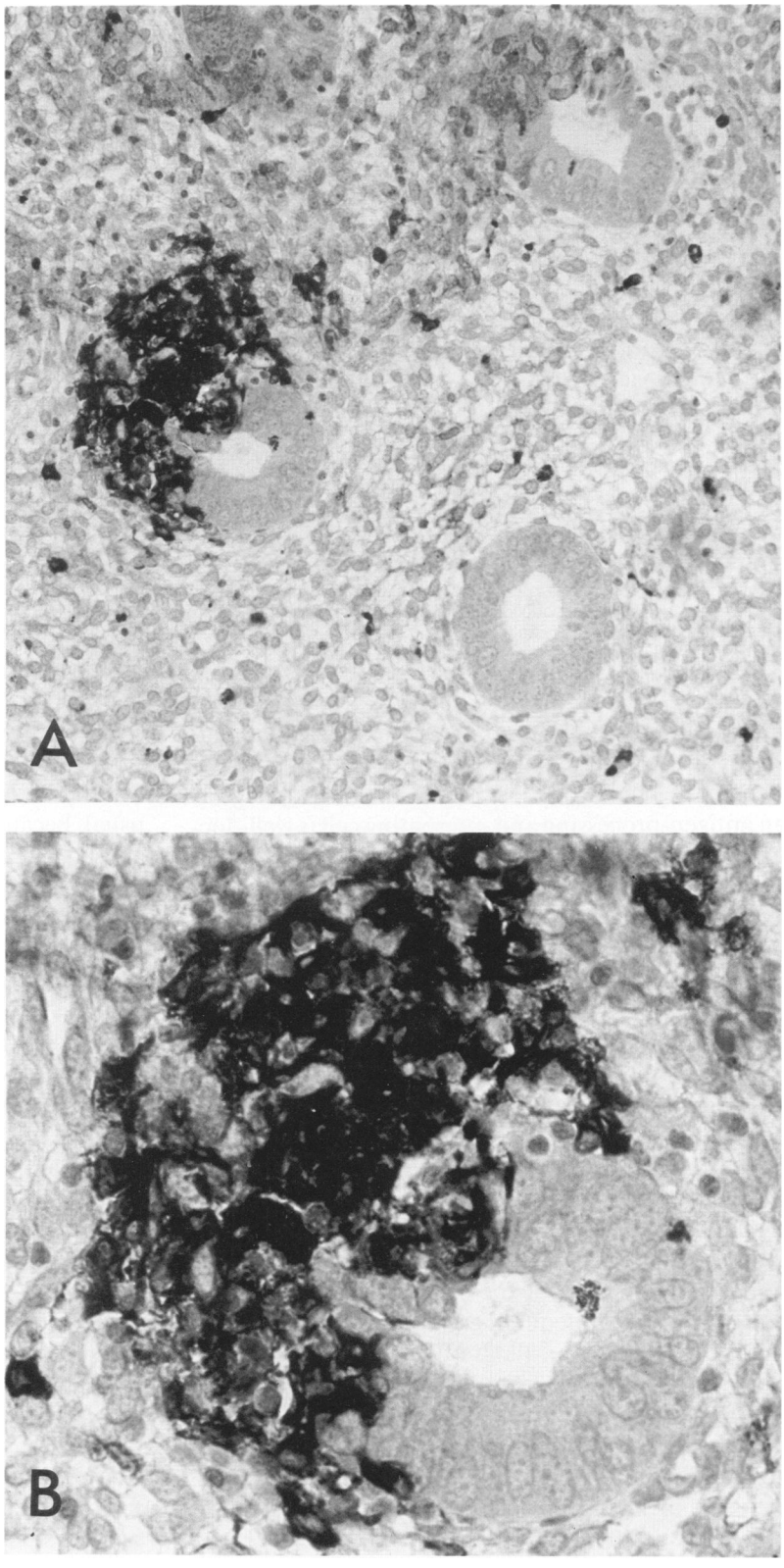

Figure 3—Macrophages were distributed both within the stromal aggregates and singly in the stroma. Note their long cellular processes and relationship to epithelium and lymphocytes. (Acid formalin-fixed paraffin section, TAL-1B5 stained, **A,** ×300; **B,** ×800)

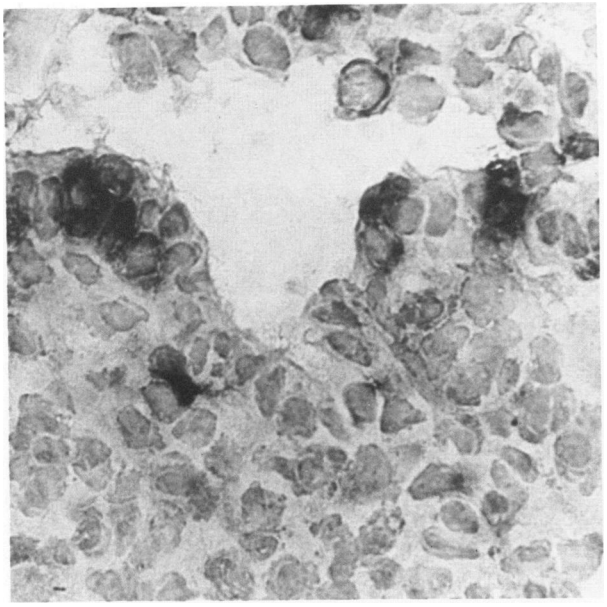

Figure 4—Scattered large mononuclear $T6^+$ cells were present in 5 cases. (Cryostat section, Na 1/34-stained, ×800)

increase in macrophages that may relate to the menstrual disintegration of the endometrium and its subsequent regeneration. The role of the macrophage as an antigen-processing and -presenting cell is well-documented, and it is interesting to observe its location, both adjacent to the epithelium and mingling with the stromal T-cell nodules. This finding has also been described by Morris et al.[15]

An observation of potential interest is the presence of large numbers of T suppressor/cytotoxic cells ($T8^+$) in the endometrium, particularly within the stromal nodules. Although significant T-cell populations have been described in many surface epithelia (skin, cervix, and gut[16–18]), the apparent morphologic predominance of T suppressor cells is unusual. While merely speculative at this time, this finding raises the possibility that these cells may play a role in the regulation of the immediate local immune response of the gestational endometrium at the early implantation stage and during the subsequent growth of the fetus, which may be regarded as an allograft. It is of interest to note that studies on the genital tract of allopregnant 3H/He J mice have revealed uterine blood and decidual lymphocytes to be a potent source of suppressor activity.[19] While suppression of maternal immune responsiveness has been postulated to contribute to tolerance of the fetal allograft,[20] the complex interactions of the local and systemic immune response are currently under extensive investigation. In addition, the role of the immune system in normal and abnormal pregnancy is also of current interest.[21] A study of these cells in spontaneous abortion and infertility may elucidate an immune contribution, if any, to these pathologic states.

The organization of immunocompetent cells within the endometrium differs in several respects from that in the gut. Although intraepithelial $T8^+$ cells appear to be analogous to those described in the gut[22] and cervix, intraepithelial B cells, as described in the "dome" region of the gut,[18] are absent. The organization of T cells and macrophages into stromal nodules has not been described in the gut. In addition, the gut contains abundant plasma cells and significant B-cell populations, both as lymphoid follicles and as lamina propria cells; these cells are rare and do not form an indigenous population in the normal endometrium, unless located within lymphoid follicles. Thus the finding of any mature plasma cells (none of which were observed in our cases) in human endometrium may indeed be indicative of a pathologic state (ie, chronic endometritis). Although we observed lymphoid follicles in only 3 cases, the relatively superficial nature of a curettage procedure may be responsible for this low incidence, because lymphoid follicles are generally located in the stratum basalis. Occasional natural killer cells were seen both in the lymphoid follicles and the stroma. This finding is not unusual, because natural killer cells may be observed in all lymphoreticular tissues. The reason for the cross-reactivity of the Leu-7 antibody with the glandular epithelium remains to be determined; it is interesting to note that epithelial staining with the Leu-7 antibody has recently been described in the normal prostate.[23]

Another finding of potential significance is the demonstration of large mononuclear $T6^+$ cells within the epithelium of 5 out of 30 cases, sometimes appearing to send dendritic cell processes among the epithelial cells. Their morphology and location, as well as the absence of staining with T/B/macrophage markers suggests that they may belong to the Langerhans/interdigitating reticulum cell series; however, we failed to observe any intraepithelial $HLA\text{-}DR^+$ cells of similar morphology in our cases. While these cells have been observed in various lymphoid tissues in the T-cell zones and in several surface epithelia, including the skin and cervix,[3,4] their definitive identification in the endometrium will depend on further studies, including extensive sampling of the endometrium, histochemistry, and immunoelectron microscopic studies, to demonstrate Birbeck granules within the $T6^+$ cells.

The use of endometrial curettage specimens only in this study has limited the observations and conclusions to the stratum functionalis of the endometrium

alone. However, it was the objective of the authors to obtain as "normal" a tissue sampling as feasible and delineate indigenous cell populations. The clinical indications for hysterectomy are generally not appropriate to such an objective, whereas the curettage specimens were obtained from tubal ligations and other indications where a reasonably "normal" leukocytic cell population could be expected. A study using selected hysterectomy specimens may supply further information about the stratum basalis.

In summary, the endometrium contains significant numbers of immunologically competent cells which may serve to maintain the normal physiologic state. The unusual finding of large numbers of $T8^+$ cells deserves further investigation under both physiologic and pathologic conditions to determine the functional contribution of these cells to the local immune response. Lastly, the definitive identification of the interdigitating reticulum cell in the endometrium would also be of interest, because they are known to participate in antigen presentation. This study has demonstrated that although lymphocytes and macrophages have long been recognized in the endometrium (Feyrtyr in 1957 labeled them "resting wandercells"), newer techniques may make possible the delineation and study of smaller constituent cell populations under various conditions and lead to a further understanding of disease processes in the future.

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